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Proteasome inhibitors in mantle cell lymphoma

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Abstract

Mantle cell lymphoma (MCL) represents a subtype of non-Hodgkin's lymphoma (NHL) which has a relatively poor prognosis compared to other forms of NHL. Despite multiple options for cytotoxic chemotherapy, attempts to prolong the survival of patients with this disease have not yet met with success. Consequently, the development of targeted approaches to therapy which minimize toxicities has potentially important implications for MCL. Proteasome inhibitors preferentially kill transformed cells through diverse mechanisms. The proteasome inhibitor bortezomib was initially approved for patients with relapsed or refractory multiple myeloma and now has been approved for relapsed or refractory MCL. The introduction of newer proteasome inhibitors with activity in bortezomib-resistant disease and reduced toxicity profiles may yield further benefits. Multiple ongoing studies are building on the known efficacy of proteasome inhibitors in MCL by evaluating combination regimens involving either cytotoxic or targeted therapies, with the ultimate goal of prolonging survival in this patient population.

Keywords

Non-Hodgkin's lymphoma; proteasome inhibitor; mantle cell lymphoma; bortezomib; carfilzomib

Mantle cell lymphoma-general considerations

Mantle cell lymphoma (MCL) is a form of non-Hodgkin's lymphoma (NHL) that exhibits characteristics of both indolent and aggressive forms of NHL. Like many of the more common indolent lymphoid neoplasms, MCL appears to be incurable with conventional chemotherapy. However, MCL does not have an indolent natural history; instead, it displays shorter disease-free and overall survivals most characteristic of aggressive lymphomas. As a consequence, currently, there is no established standard of care for this disorder, and tangible evidence of improved survival in MCL with new therapies has largely been lacking. In the absence of universally accepted standard management in MCL, it is generally thought that patients with this disease should be referred for participation in prospective clinical trials. Very few patients present with localised MCL, and the available published literature on management of this disorder is for the most part retrospective and anecdotal [1, 2].

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The large majority of patients with MCL have advanced-stage disease and require systemic therapy. For patients who do not have access to clinical trials for first-line therapy, several regimens have shown significant activity, including R-Hyper-CVAD (rituximab, cyclophosphamide, vincristine, doxorubicin and dexamethasone) alternating with methotrexate and cytarabine, R-CHOP, and R-EPOCH (rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin). Despite initial responses in a substantial fraction of patients, relapse commonly occurs, within a median interval of 15–18 months in the absence of adjuvant stem cell transplantation (autologous or allogeneic). As in the case of initial treatment, the optimal approach to the management of relapsed or recurrent disease remains to be defined. For this reason, entry of patients into clinical trials is also strongly encouraged under these circumstances. In light of these considerations, MCL appears to represent an attractive setting for trials involving novel targeted agents [3–8].

Proteasome inhibitors-background

A role for single agents, such as the proteasome inhibitor bortezomib, for relapsed and refractory MCL, has now been firmly established [9–11]. The ubiquitin-proteasome pathway is responsible for the degradation of diverse intracellular proteins. However, in addition to control of protein turnover, it also plays an important role in multiple critical processes, including responses to various cellular stresses (e.g., oxidative injury and DNA damage), maintenance of the balance between pro- and anti-apoptotic proteins, and regulation of signal transduction, among numerous others [12]. Following sequential ubiquitination by an E1 ubiquitin-activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin ligase, unwanted or misfolded proteins are channelled into the proteasome complex, consisting of 20S and 19S or 11S components, where they are targeted for degradation [13]. The proteasome complex exhibits chymotryptic, tryptic, and caspase-like activities, although immunoproteasome activity has recently been described [14]. Chymotryptic activity is thought to represent the primary target of proteasome inhibitors (e.g., bortezomib), although other activities may also be inhibited by this or related agents [15].

Because the proteasome is ubiquitous in eukaryotic cells, and is involved in numerous critical cellular functions, its viability as a therapeutic target was initially questioned. However, contrary to expectations, proteasome inhibitors were shown in preclinical studies to be selectively toxic toward transformed cells, and to circumvent resistance to cell death stemming from over-expression of anti-apoptotic proteins such as Bcl-2 [16]. In retrospect, this selectivity may reflect the dependence of transformed cells on systems capable of protecting them from various oncogenic stresses, including proteotoxic stress [17]. In this context, the observation that tumours characterised by extensive protein production and secretion (e.g., multiple myeloma) are particularly susceptible to proteasome inhibitors is more readily understandable.

Proteasome inhibitors-mechanisms of action

Proteasome inhibitors are pleiotropic agents in that their lethality may reflect diverse actions, including those that may be independent of proteasome inhibition. It is important to understand the mechanisms by which these agents kill transformed cells, as such information can provide a foundation for rational combination strategies. A summary of mechanisms implicated in proteasome inhibitor lethality follows below.

- a. Accumulation of misfolded proteins. Transformed cells are often characterised by increased protein turnover, which leads to various forms of proteotoxic stress, including endoplasmic reticulum stress. Disruption of the degradation of unfolded proteins elicits the unfolded protein response, which in its initial stages may be cytoprotective, but which at later stages becomes pro-apoptotic [18]. Induction of

the pro-apoptotic arm of the unfolded protein response and induction of endoplasmic reticulum (ER) stress has been invoked as a mechanism of proteasome inhibitor lethality [19, 20].

- b.** Oxidative injury. Proteasome inhibitors such as bortezomib have been shown to induce accumulation of reactive oxygen species (ROS) in both solid tumour [21] as well as malignant hematopoietic cells [22]. The ability of antioxidants to block proteasome inhibitor lethality argues for an important role for oxidative injury in proteasome inhibitor lethality. In this context, recent studies in MCL suggest that antioxidant defences may represent a critical mediator of bortezomib resistance [23].
- c.** Inhibition of NF- κ B. By blocking degradation of the NF- κ B-inhibitory protein I κ B α , proteasome inhibitors block transcription of NF- κ B-dependent anti-apoptotic genes [24]. However, this notion has recently been called into question, and the capacity of proteasome inhibitors to block NF- κ B activation may not be universal [25].
- d.** Up-regulation of pro-apoptotic proteins. Disruption of proteasome function can result in accumulation of pro-apoptotic proteins such as Bim [26], shifting the balance from cell survival to cell death.
- e.** Stabilisation of p53. Proteasome inhibitors can induce accumulation of p53, which in turn may lead to promotion of apoptosis [27].
- f.** Stabilisation of JNK. Interference with proteasome function can lead to accumulation of the stress-related kinase JNK, which generally plays a pro-apoptotic role [28].
- g.** Interference with DNA repair. Participation of the proteasome in DNA repair processes may lead to potentiation of DNA damage by bortezomib [29].
- h.** Anti-angiogenic effects. The ability of proteasome inhibitors to interfere with angiogenesis may contribute to *in vivo* anti-tumour activity [30].

A summary of these and other candidate mechanisms of proteasome inhibitor lethality is illustrated in Figure 1.

Proteasome inhibitors in the clinic

The boronic anhydride bortezomib was the first of the proteasome inhibitors to enter the clinical arena [31]. Since then, multiple other proteasome inhibitors have been developed with 3 major goals in mind: 1) circumventing bortezomib resistance; and 2) ameliorating some of the dose-limiting toxicities of bortezomib e.g., neurotoxicity; and 3) feasibility of oral administration. For example, MLN-9807 is, like bortezomib, a reversible proteasome inhibitor, but in contrast to bortezomib, is orally available, and purportedly has less neurotoxicity. CEP-18770 has similar characteristics [15]. Carfilzomib (previously known as PR-171) is an irreversible epoxy-ketone proteasome inhibitor that in preclinical studies has shown activity against bortezomib-resistant multiple myeloma (MM) cells [32]. In addition, NPI-0052 is an irreversible, non-peptide proteasome inhibitor that is active against all 3 proteasome activities [33]. NPI-0052 acts by covalently modifying active site threonine residues of the 20S proteasome and is orally bioactive [34]. In contrast to bortezomib, experience with the latter proteasome inhibitors in MCL is currently limited.

Proteasome inhibitors in mantle cell lymphoma - preclinical data

One of the initial preclinical studies to demonstrate activity of proteasome inhibitors in MCL was that of Perez-Galan et al., who reported that bortezomib, administered at very low concentrations, potently induced apoptosis in MCL cell lines as well as primary samples in association with ROS generation and activation of both Bax and Bak [35]. Of note, lethality was associated with pronounced up-regulation of the pro-apoptotic protein Noxa, particularly in cells with functional p53. It was also shown that Noxa prevented up-regulation of Mcl-1, leading to release of Bak from this protein, culminating in apoptosis. Induction of Noxa by bortezomib in MCL cells has been shown to be independent of NF- κ B and AKT [36].

This group subsequently reported that the BH3-mimetic GX15-070 (obatoclax) interacted synergistically with bortezomib to induce cell death in MCL cells through a mechanism also involving neutralisation of Mcl-1 accumulation, and displacement from and activation of Bak [37]. The result was activation of the caspase cascade and induction of mitochondrial injury and apoptosis in both cell lines and primary MCL cells. Subsequently, it was reported that the BH3-mimetic ABT-737 interacts synergistically with bortezomib in MCL cells as well as diffuse large B-cell lymphoma (DLBCL) cells [38].

Sequence-dependent synergism in MCL cell lines has been described under conditions when bortezomib is administered after the nucleoside analogue ara-C [39]. Interestingly, promising results were reported in MCL patients treated with these agents [29]. *In vitro* and *in vivo* synergism in MCL cell lines has also been described between bortezomib and rituximab or cyclophosphamide [40].

Synergism between proteasome and histone deacetylase (HDAC) inhibitors has been described in various malignant hematopoietic cell types, including leukaemia and MM [41, 42]. Multiple mechanisms have been invoked to explain this phenomenon, including inhibition of NF- κ B, disruption of aggresome function, and induction of ER stress, among others [43]. In accord with these observations, synergistic interactions between the HDAC inhibitor vorinostat and proteasome inhibitor bortezomib was reported in multiple MCL cell lines [44]. Parallel results were obtained with a combination of the class I HDAC inhibitor romidepsin and bortezomib [45]. Synergism between the HDAC inhibitor panobinostat and bortezomib in MCL lines has been related to induction of pro-apoptotic components (e.g., CHOP) of the ER stress response [46].

A two-pronged approach to circumventing proteasome inhibitor resistance in MCL would involve the use of an irreversible proteasome inhibitor such as carfilzomib in combination with an HDAC inhibitor. Indeed, synergistic effects were observed with the combination of vorinostat and carfilzomib in both GC- and ABC-DLBCL cells *in vitro* and *in vivo*, including those resistant to bortezomib [47]. Subsequently, parallel results were reported in MCL cell lines [48]. Notably, enhanced lethality was observed for the carfilzomib/vorinostat regimen in *in vivo* MCL xenograft models, and in MCL cells resistant to bortezomib.

In addition to standard mechanisms of resistance to proteasome inhibitors (e.g., up-regulation or mutation of proteasome sub-units) [12], several novel mechanisms have been implicated in bortezomib resistance in MCL cells. For example, plasmacytic differentiation has been reported to protect MCL cells from bortezomib lethality [49]. Furthermore, HSP90 antagonists have been shown to overcome bortezomib resistance mediated by the ER chaperone protein BiP/Grp78 [23]. As noted, resistance to oxidative stress has been found to protect MCL cells from bortezomib lethality [23].

Proteasome inhibitors in mantle cell lymphoma - clinical status

Phase I and II clinical studies have demonstrated that bortezomib is a well-tolerated agent with minimal haematologic toxicity in MCL. Approval of bortezomib was based on the results of the phase II multicenter PINNACLE study as well as additional data from 4 other relevant phase II studies [9–11, 50–52] (table 1).

For results involving bortezomib as a single agent, the overall response rate in smaller trials ranged from 29–50%, with complete response rates ranging from 4–21%.

In the PINNACLE study, 155 patients were enrolled, and of these, 141 were evaluable. Patients were treated with bortezomib 1.3 mg/m² administered on days 1, 4, 8, and 11 of a 21-day cycle, and received up to 17 cycles. The overall response rate was 33%, with an 8% complete response or unconfirmed complete response rate. Median time to progression (TTP) was 6.2 months, and median duration of response (DOR) was 9.2 months. The median overall survival (OS) was not reached after a median follow-up of 13.4 months. Patients included in this study had received 1–3 prior regimens. The most common adverse events of grade 3 or higher included peripheral neuropathy (13%), thrombocytopenia (11%), and fatigue (12%); death attributed to study treatment occurred 3% of patients [9]. These findings were consistent with adverse events reports for MM trials involving bortezomib [53, 54].

Updated time-to-event data of the PINNACLE study with an extended median follow-up of 26.4 months confirmed the high activity of bortezomib in relapsed or refractory MCL patients. The median OS was 23.5 months, and the median TTP was 6.7 months. In responding patients, median TTP was 12.4 months, median DOR was 9.2 months, median OS was 35.4 months, and the one-year survival rate was 69% overall and 91% in responders. Median OS from diagnosis was 61.1 months after a median follow-up of 63.7 months [52].

Bortezomib is currently indicated in patients with MCL who experience disease progression after 1 prior therapy. The dose generally employed for single agent bortezomib in patients with relapsed or refractory MCL is 1.3 mg/m² as an intravenous (IV) push on days 1, 4, 8, and 11 of a 21 day cycle. Subcutaneous (SC) administration of bortezomib has been investigated in 2 clinical trials in multiple myeloma, including MMY-3021, a phase III, randomised, open-label, prospective study of SC versus IV administration of bortezomib in patients with relapsed MM [55]. SC administration of bortezomib appeared to have an improved safety profile compared with IV administration. The long term outcome of bortezomib-mediated peripheral neuropathy has not yet been investigated in MCL. Clinical trial results for newer proteasome inhibitors which reportedly exhibit less neurotoxicity than bortezomib may help to address this issue.

Preclinical findings suggest synergistic or additive effects with various cytotoxic or targeted therapies. Multiple phase I and phase II clinical trials are currently addressing the role of bortezomib as a component of cytotoxic chemotherapeutic regimens in newly diagnosed, as well in relapsed/refractory MCL patients. In addition, the role of maintenance therapy with bortezomib in these settings is also being investigated.

A multi-institutional, randomised, phase III study is currently evaluating R-CHOP versus R-CHP-bortezomib in patients with newly diagnosed MCL [56, 57]. In addition, multiple trials are evaluating various cytotoxic chemotherapeutic combinations with bortezomib. These include Hyper-CVAD [58], R-EPOCH, R-CHOP, and consolidation and maintenance post-R-CHOP induction.

In an observational study of high dose cytarabine, dexamethasone, and bortezomib in relapsed/refractory MCL, the overall response rate was 50%, with a complete response rate of 25%, and median overall survival of 15.5 months in 8 heavily pretreated patients. Significant (grade 3 and higher) haematological toxicities were reported [59].

Results of a phase I dose escalation study involving a combination of bortezomib and rituximab, in which 2 patients with MCL were treated, raised the potential concern that rituximab may exacerbate bortezomib-associated peripheral neuropathy [60]. An ongoing phase II study is evaluating clinical responses to and tolerability of the combination of bortezomib and rituximab in relapsed/refractory MCL [61].

The combination of ibritumomab monoclonal antibody radioimmunotherapy with bortezomib resulted in an overall response rate of 56%, with a complete response rate of 33% and overall survival of 80% in 9 patients [62].

With the development of novel targeted therapies, several new combination regimens for patients with relapsed/refractory MCL are being explored. Numerous preclinical studies have demonstrated synergistic interactions between proteasome and HDAC inhibitors. In this context, a phase II trial of bortezomib and vorinostat for patients with MCL or DLBCL has been initiated. Notably, 17 bortezomib-naïve, relapsed/refractory MCL patients were treated with this regimen, with preliminary overall response rates of 47% [63].

The novel pan Bcl-2 inhibitor obatoclax mesylate (GX15-070) is currently being evaluated in combination with bortezomib in a phase I dose escalated trial. Thrombocytopenia has been the most common 3/4 grade adverse event thus far [64].

On the clinicaltrials.gov website, 75 combination therapy clinical trials with bortezomib in MCL are currently registered.

As noted above, experience with second-generation proteasome inhibitors in MCL is limited. However, the activity of the irreversible proteasome inhibitor carfilzomib in malignant lymphoid cells resistant to bortezomib makes it an attractive candidate for combination regimens. The rationales for including carfilzomib in such multi-agent regimens include: a) diminished neurotoxicity; b) the potential for irreversible proteasome inhibition; c) tolerability of a more chronic administration schedule compared to bortezomib; and d) preclinical and clinical evidence of activity in the bortezomib-resistant setting. In this context, a phase I trial of carfilzomib and vorinostat in patients with refractory NHL, including MCL, has recently been initiated.

Conclusions

Proteasome inhibitors, and more specifically bortezomib, have a clearly established role in the treatment of MCL. The current challenge will be to build upon the success of single agent activity of bortezomib in this disease. Several key questions remain to be addressed in the future. First it will be important to determine whether bortezomib has a role to play in MCL in combination with standard chemotherapy (e.g., R-CHOP), as has recently been suggested in the case of ABC-DLBCL [65]. Analogously, the role of bortezomib or other proteasome inhibitors as maintenance therapy in MCL remains to be established. In addition, it will be critical to determine whether the theoretical advantages of second-generation proteasome inhibitors (e.g., diminished neurotoxicity, activity in bortezomib-resistant cells, and ease of oral administration) offer tangible clinical benefits. Furthermore, it will be important to identify, in addition to standard cytotoxic agents, the optimal targeted agents to be combined with proteasome inhibitors for the treatment of patients with MCL. Currently, the bulk of preclinical data suggest HDAC inhibitors or BH3-mimetics may be

particularly promising, but numerous other possibilities exist. Finally, it will be critical to determine whether second-generation proteasome inhibitors will prove superior to bortezomib in such novel combination strategies. Answers to these questions will hopefully lead to more effective proteasome inhibitor-based strategies in MCL treatment in the future.

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Figure 1.

Candidate mechanisms of proteasome inhibitor lethality. ROS = reactive oxygen species; UPR = unfolded protein response; DNMT1 = DNA methyltransferase 1.

Table 1
Response to bortezomib in patients with relapsed/refractory mantle cell lymphoma.

Reference	N	ORR (%)	CR (%)	Median OS (Months)	Median response duration (months)
Fisher et al.	141	33	8	23.5	9.2
Goy et al.	29	41	21	NR	NR
Strauss et al.	24	29	4	NR	NR
O'Connor et al.	10	50	10 *	NR	NR

ORR - overall response rate, CR - complete response, OS - overall survival, * - complete response unconfirmed, NR - not reported